Variable Glyceryl Dinitrate Formation Following Infusions of Glyceryl Trinitrate at Different Vascular Sites in the Rat

Emi Nakashima,[1,2] David T.-W. Lau,[1] and Leslie Z. Benet[1,3]

Received August 22, 1990; accepted January 31, 1991

The availability of glyceryl trinitrate (GTN) and the differential formation of dinitrate metabolites (GDNs) in various organs as a function of routes of administration were investigated in the rat. GTN was infused at 2.0 μg/min via the left femoral vein (LFV), left external jugular vein (LJV), left femoral artery (LFA), and hepatic portal vein (HPV). Blood concentrations of GTN and GDNs were measured in femoral arterial samples. Different infusions yielded GTN steady-state concentrations in the following rank order: LJV ≥ LFV > LFA ≥ HPV. Furthermore, the GDN formation ratios (1,2-GDN/1,3-GDN) are different: LFV ≥ LJV > LFA > HPV. The availabilities of GTN through the leg, vein, and liver were derived. GTN is significantly extracted and metabolized in these organs, and the leg and the vein prefer 1,2-GDN formation, while the liver forms 1,3-GDN predominantly.

KEY WORDS: nitroglycerin; glyceryl; nitrate; clearance; metabolism; uptake.

INTRODUCTION

Glyceryl trinitrate (GTN), more commonly known as nitroglycerin, is one of the most frequently prescribed drugs for the management of angina pectoris. GTN has been formulated into different dosage preparations with varying onset times and duration of action, which allows it to be used in either acute or prophylactic therapy. GTN exhibits unusual pharmacokinetic properties, but it was not until the last decade, with the development of sensitive and specific methodologies for assaying GTN and its dinitrate metabolites (GDNs), that these pharmacokinetic abnormalities were confirmed.

GTN has been reported to possess a clearance that approximates or even exceeds the cardiac output, both in rats (1) and in man (2,3). The drug can be metabolized by different organ homogenates (4,5) and in blood (6,7). Significant extraction of GTN by various organ beds has been reported in sheep (8). Furthermore, it has been demonstrated that GTN can be extensively taken up by vascular tissues (5). According to the generally recognized mechanism of action of organic nitrates, biotransformation of these chemicals, following vascular uptake, is essential for the generation of vasodilating effects (9). Following GTN administration, the drug is rapidly denitrated to form its dinitrate metabolites: 1,2- and 1,3-glyceryl dinitrates (1,2-GDN and 1,3-GDN). Recently, results from our laboratory demonstrated that the pattern of glyceryl dinitrate formation may be a function of the route of administration (10). However, a confounding variable was present in the study. Usually, when GTN is administered via various routes of administration, different doses of the drug are used. Hence the results may be interpreted as a dose dependency, instead of a route dependency, of selective GDN formation. In this study, GTN infusions of equivalent rates were administered to rats via the following vascular sites: left femoral vein (LFV), left jugular vein (LJV), left femoral artery (LFA), and hepatic portal vein (HPV). The objective of this study was to elucidate the extraction of GTN and the pattern of GDN formation in selected organs which have been shown to be important for GTN metabolism.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (256 ± 5 g; $n = 5$ for each experimental group) were purchased from Bantin-Kingman (Fremont, CA) and were housed in the Animal Care Facility of the University of California at San Francisco for at least 2 days prior to the experiment. The rats were housed in facilities maintained at 12-hr cycles of alternating light and dark periods. The animals were allowed free access to water and laboratory Purina Chow (Ralston Purina Co., Richmond, IN) until the day of experimentation.

Chemicals

GTN was purchased as 10-ml vials of Tridil (5 mg/ml) from DuPont Pharmaceuticals (Wilmington, DE). 1,2- and 1,3-GDNs (>99% purity) were graciously supplied by Marion Laboratories (Kansas City, MO). The organic solvents used in the extraction procedure, i.e., pentane, methyl-*t*-butyl ether, and butyl acetate, were purchased at the highest grade available from EM Sciences (Cherry Hill, NJ).

Surgical Procedures

For induction of anesthesia, the rats received a 50-mg/kg i.p. injection of pentobarbital sodium, obtained as Nembutal solution from Abbott Laboratories (North Chicago, IL). Subsequent maintenance doses of 5 mg/kg i.p. were given every 30 min throughout the duration of the pharmacokinetic studies. A sufficient depth of anesthesia was judged to have been attained when the corneal reflex and response to hind-limb pinch were no longer elicitable.

The right femoral artery of the animal was exposed and catheterized with PE-50 polyethylene tubing (Clay Adams, Parsippany, NJ) to enable arterial blood sampling. For the purpose of GTN infusions via different sites, a piece of PE-10 polyethylene tubing (Clay Adams) was placed in one of the following locations of the rat: (a) the left femoral vein (for LFV infusions), (b) the left external jugular vein, toward the direction of the heart (for LJV infusions), (c) the left femoral artery (for LFA infusions), with the cannula placed in the

[1] Department of Pharmacy, School of Pharmacy, University of California, San Francisco, California 94143-0446.
[2] Present address: Department of Hospital Pharmacy, Kanazawa University, Takara-machi, Kanazawa 920, Japan.
[3] To whom correspondence should be addressed.

0724-8741/91/0700-0877$06.50/0 © 1991 Plenum Publishing Corporation

direction away from the descending aorta, and (d) the hepatic portal vein (for HPV infusions). A scheme representing the sites of GTN administration and blood sampling used in the experiment is depicted in Fig. 1.

All catheters were kept patent with heparin sodium (20 IU/ml; Elkins-Sim Inc., Cherry Hill, NJ). The surgical wounds were covered with gauze kept moist with normal saline in order to minimize tissue fluid loss. Maintenance doses of pentobarbital sodium, as 5-mg/kg i.p. injections, were given every 30 min throughout the duration of the pharmacokinetic study. Rectal temperatures were monitored and maintained at 38 ± 1°C using an incandescent lamp.

Blood Sampling and Analysis

GTN infusions were administered at a rate of 2.0 μg/min using a Harvard Infusion/Withdrawal Pump (Millis, MA). The flow rate was maintained at 0.0136 ml/min for 60 min. The pump was calibrated previously and found to exhibit a coefficient of variation of 2.0% at the above infusion rate. Femoral arterial blood samples (100 μl) were collected at 30, 40, 50, 60, 62, 65, 70, 75, and 80 min after the initiation of GTN infusion. The blood samples were immediately frozen by pipeting into silanized glass tubes that were placed upon a mixture of dry ice and methanol. The whole process of blood sampling took less than 10 sec in order to minimize the effects of blood metabolism. Based on our previous experience with human blood, the degradation half-life for GTN was approximately 20 min at 37°C (6). It is unlikely that the short blood sampling procedure could affect the GTN and GDN levels significantly. Blood samples were then stored at −80°C until analysis. Blood concentrations of 1,2- and 1,3-GDNs were analyzed using a specific and sensitive capillary column gas chromatography procedure previously developed in our laboratory, with slight modification (11). Briefly, 3 × 10-ml mixtures of pentane and methyl-*t*-butyl ether (80:20%) were used to extract the samples instead of a mixture of pentane and methylene chloride. 2,6-Dinitrotoluene (10 ng) was used as the internal standard. Standard curves of GTN and the GDNs show linearity from 0.5 to 20 ng per sample.

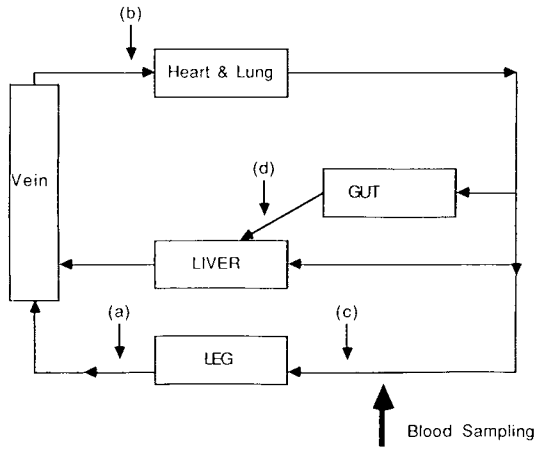

Fig. 1. A simplified scheme of the sites of GTN infusion and blood sampling: (a) left femoral vein infusion (LFV); (b) left external jugular vein infusion (LJV); (c) left femoral artery infusion (LFA); (d) hepatic portal vein infusion (HPV). Blood samples were consistently drawn from the right femoral artery for all sites of infusions.

Pharmacokinetic and Statistical Analysis

The apparent clearances (CL_{app}) of GTN for the various routes of administration are calculated by dividing the rate of GTN infusion (R) by the steady-state concentrations (C_{ss}) of GTN (determined as the mean of the 40-, 50-, and 60-min GTN measurements). For example, for the left femoral vein infusions,

$$CL_{app,GTN,LFV} = R/C_{ss,GTN,LFV} \quad (1)$$

Similarly, the apparent GTN clearances for the other three infusion sites can be calculated. The availabilities of GTN through different organs (F_{Organ}) can be calculated as a ratio of the apparent clearances, which are related to the ratio of steady-state concentrations of GTN following different routes of administration:

$$F_{LEG} = CL_{app,LFV}/CL_{app,LFA} = C_{ss,GTN,LFA}/C_{ss,GTN,LFV} \quad (2a)$$

$$F_{LIVER} = CL_{app,LFV}/CL_{app,HPV} = C_{ss,GTN,HPV}/C_{ss,GTN,LFV} \quad (2b)$$

$$F_{VEIN} = CL_{app,LJV}/CL_{app,LFV} = C_{ss,GTN,LFV}/C_{ss,GTN,LJV} \quad (2c)$$

Based on the mass balance principle, which assumes that the amount of metabolite formed must equal the amount of metabolite that is eliminated, the measurable formation clearances of each GDN from GTN are estimated by the following equations:

$$CL_{f,1,2\text{-GDN}} \times C_{ss,GTN}/MW_{GTN} = CL_{1,2\text{-GDN}} \times C_{ss,1,2\text{-GDN}}/MW_{1,2\text{-GDN}} \quad (3a)$$

$$CL_{f,1,3\text{-GDN}} \times C_{ss,GTN}/MW_{GTN} = CL_{1,3\text{-GDN}} \times C_{ss,1,3\text{-GDN}}/MW_{1,3\text{-GDN}} \quad (3b)$$

where the CL_f's represent the formation clearance of the individual GDNs. $CL_{1,2\text{-GDN}}$ and $CL_{1,3\text{-GDN}}$ are the mean clearances (elimination) of the GDNs, determined for three groups of rats in a companion study (12) following i.v. infusions of each GDN ($CL_{1,2\text{-GDN}}$ = 27.6 ml/min/kg, $CL_{1,3\text{-GDN}}$ = 21.6 ml/min/kg). $C_{ss,1,2\text{-GDN}}$ and $C_{ss,1,3\text{-GDN}}$ are measured (average of 50- and 60-min GDN concentration values). MW_{GTN} and MW_{GDN} are the molecular weights of GTN (227 D) and the GDNs (182 D).

The percentage of GTN clearance that can be accounted for by GDN formation (% recovery) is calculated as follows:

$$\%\ \text{recovery} = (CL_{f,1,2\text{-GDN}} + CL_{f,1,3\text{-GDN}})/CL_{app,GTN} \quad (4)$$

where $CL_{app,GTN}$ is the apparent clearance of GTN for a particular route of administration.

The four experimental groups were compared using the one-way analysis of variance. The Newman–Keuls test was performed for the purpose of multiple comparisons between individual groups, as described in Ref. 13. Statistical significance is achieved at $P < 0.05$.

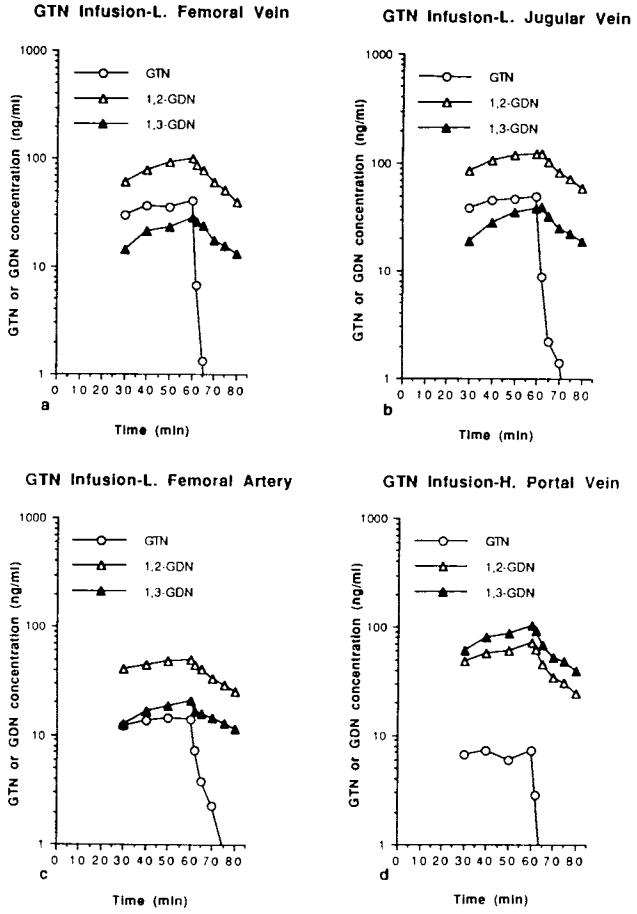

Fig. 2. Blood concentration (ng/ml) profile of GTN and GDNs following GTN infusions (2.0 μg/min for 60 min) via (a) left femoral vein, (b) left jugular vein, (c) left femoral artery, and (d) hepatic portal vein.

RESULTS

The concentrations of GTN, 1,2-GDN, and 1,3-GDN following the four different routes of GTN infusion at 2.0 μg/min are depicted in Fig. 2. It is evident that there are differences in the steady-state concentrations of GTN, indicating significant GTN extraction through various organ beds. Moreover, there are also differences as to the preferential formation of GDN metabolites. This suggests that different organ beds which extract and metabolize GTN may have different selective formation of the GDNs. Table I shows the steady-state concentrations of GTN (average of the 40-, 50-, and 60-min time points) and GDNs (average of the 50- and 60-min time points). LFV and LJV infusions resulted in significantly higher GTN steady-state concentrations than the LFA and HPV infusions. Furthermore, the 1,2-GDN concentrations resulting from the LFV and LJV infusions exceeded those for LFA and HPV infusions. HPV produced the highest 1,3-GDN concentration, which was significantly higher than 1,3-GDN concentrations following LFV, LJV, and LFA infusions, which were all comparable. The GDN ratios resulting from the various GTN infusions were calculated as 1,2-GDN divided by 1,3-GDN concentrations. Figure 3 demonstrates that LFV and LJV infusions produced significantly higher GDN ratios than LFA infusions. Comparing these infusions to the HPV infusions, however, the selectivity of 1,2-GDN over 1,3-GDN formation was reversed when GTN was infused via the hepatic portal vein.

The apparent clearance values for GTN following various sites of infusions are calculated as described under Materials and Methods and are listed in Table II. The apparent GTN clearances of the LFV and LJV infusions are not significantly different and are comparable to the cardiac output of the rat. On the other hand, the apparent GTN clearances of the LFA and HPV infusions are much higher than the cardiac output, indicating significant first-pass GTN extraction, probably by the leg and the liver. Using the clearance values of the GDNs in rats, as recently reported by our laboratory (12), the formation clearances of the GDNs are calculated. The percentage of GTN clearance accounted for by measurable GDN formation for the LFV, LJV, and HPV infusions is about 60%, whereas the value for LFA infusions is approximately 30%. The GTN availabilities were calculated by Eq. (2), yielding the following values: liver, 18.3%; leg (hind-limb), 37.5%; and veins, 79.1%. The liver preferentially forms 1,3-GDN, while it appears that the rest of the body forms 1,2-GDN predominantly.

DISCUSSION

Although GTN has been used as an antianginal agent for over a century, there are still many questions relating to the pharmacokinetics, pharmacodynamics, and mechanism of action of the drug. Elucidation of the pharmacokinetic–pharmacodynamic relationship of GTN has been hindered

Table I. Steady-State Concentrations of GTN (Average of 40, 50, and 60 min) and GDNs (Average of 50 and 60 min) After GTN (2.0-μg/min) Infusions (Mean ± SD; n = 5)

Site of infusion	GTN (ng/ml)[a]	1,2-GDN (ng/ml)[b]	1,3-GDN (ng/ml)[c]	Ratio of 1,2-/1,3-GDN[d]
LFV (left femoral vein)	37.3 ± 11.2	95.7 ± 27.2	25.9 ± 12.5	4.02 ± 0.85
LJV (left jugular vein)	47.2 ± 8.7	121.1 ± 16.1	36.9 ± 10.1	3.41 ± 0.62
LFA (left femoral artery)	14.0 ± 3.8	49.2 ± 9.4	19.4 ± 4.9	2.59 ± 0.30
HPV (hepatic portal vein)	6.82 ± 6.1	66.4 ± 20.2	94.8 ± 16.3	0.73 ± 0.28

[a] Results of multiple-comparison test: LJV = LFV > LFA = HPV.
[b] Results of multiple-comparison test: LJV = LFV > HPV = LFA.
[c] Results of multiple-comparison test: HPV > LJV > LFV = LFA.
[d] Results of multiple-comparison test: LFV = LJV > LFA > HPV.

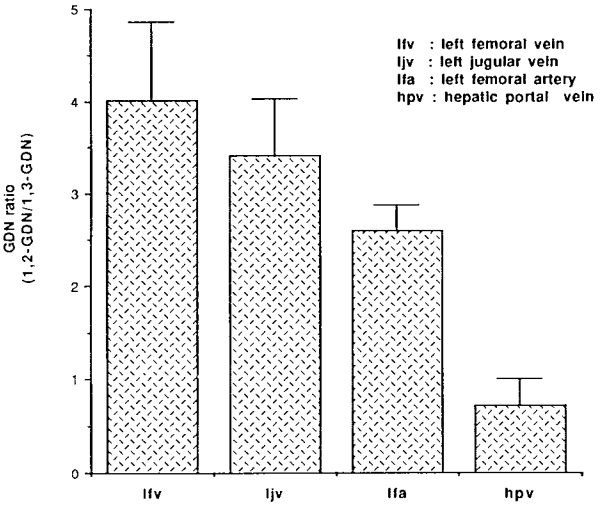

Fig. 3. GDN ratios (1,2-GDN/1,3-GDN) after GTN infusions (2.0 μg/min) at various vascular sites. Results of multiple-comparison analyses: LJV = LFV > LFA > HPV ($P < 0.05$).

by the presence of active metabolites, tolerance development, and large intra- and interindividual variability (14). GTN is now believed to exert its vasodilating actions by serving as a prodrug that can deliver the active species (an *S*-nitrosothiol, or nitric oxide) to vascular smooth muscle cells, which will then lead to stimulation of soluble guanylate cyclase and subsequent vasorelaxation (9). It is interesting to note that GTN metabolism, by which the active species are released, is essential for the generation of pharmacological effects, as demonstrated by Brien *et al.* (15). Elucidation of the metabolic profile may be essential for understanding the disposition and effectiveness of GTN. Yet only limited information on the selective metabolite formation by various organ beds under *in vivo* conditions is available. Although *in vitro* metabolic studies have been performed in homogenates, it has been demonstrated that organ homogenates may give metabolite profiles different from that observed in intact organs (16).

In this study, GTN metabolism and GDN metabolite formation in various organ beds of the rat were investigated. Since the blood samples were collected at a fixed location, i.e., the right femoral artery, in all four experimental groups, comparisons of GTN and GDN concentrations following various routes of infusions allow examination of GTN availability (or extraction) and the preference of dinitrate formation for different organs. For example, comparing LFA and LFV infusions will provide estimates for the leg; similarly, comparisons between LJV and HPV infusions with LFV infusions will give information about the venous bed (mainly vena cava) and the liver, respectively. The dinitrate metabolite ratios were determined as the ratio of 1,2- to 1,3-GDN. In some animals, it appeared that the blood concentrations were still increasing at the end of infusion. However, the half-lives of the GDN metabolites have been found to be similar in both our human (10) and our rat (12) studies. Hence, although true steady states for the GDNs may not have been reached in some experiments, the GDN ratios should still reflect the differences in the formation of the two GDNs, rather than their elimination.

The rank order of the GTN steady-state concentrations following the four sites of infusions are LJV $\geq$ LFV > LFA $\geq$ HPV. Availabilities of GTN through various organ beds were calculated as described under Materials and Methods and are shown in Table II. It is evident that all three of the organ beds investigated can extract and metabolize GTN, but in different fashion, in terms of both organ availability and the selective GDN formation. From comparisons between LFV and HPV, the availability of GTN through the liver is approximately 18%. We and others (17–20) have reported negligible or very low GTN plasma levels following oral GTN doses in man. Fung and coworkers (1) demonstrated that when higher oral doses of GTN were given to rats, the oral bioavailability was increased. A previous GTN infusion study in sheep by Cossum *et al.* (8) reported liver GTN availability measurements (0.17–0.24) similar to the values found here at comparable GTN concentrations. It should also be noted that in this study and that of Cossum *et al.* (8), GTN was administered via the hepatic portal vein and, therefore, may yield different results from oral GTN dosing in that gastrointestinal GTN metabolism may have been avoided.

It is also interesting to note that only the HPV infusions yield 1,3-GDN concentrations higher than 1,2-GDN (Table I, Figs. 2 and 3). In an *in vitro* study of GTN metabolism (21), liver homogenates of various species were compared in terms of their GDN metabolite production patterns. The rat liver is unique when compared to livers of rabbits, cats, and dogs, producing 1,3-GDN as the predominant product, whereas all the other species preferentially form 1,2-GDN at ratios exceeding 2:1. The species difference of dinitrate formation is an interesting phenomenon. Recently, studies with subcellular fractions of rabbit livers have been performed in

Table II. Metabolite Ratios and Formation Clearances of the GDNs After GTN Infusions (2.0 μg/min) via Different Sites (Mean ± SD; $n = 5$, except in HPV group, $n = 4$)[a]

Site of infusion	[1,2-GDN]/[GTN][b]	[1,3-GDN]/[GTN][b]	CL app, GTN (ml/min)[b]	CL f,1,2-GDN (ml/min)[b]	CL f,1,3-GDN (ml/min)[b]	Recovery (%)[c]
LFV (left femoral vein)	2.61 ± 0.38	0.72 ± 0.24	57.9 ± 17.8	22.9 ± 3.3	4.7 ± 1.3	51.2 ± 16.3
LJV (left jugular vein)	2.60 ± 0.30	0.77 ± 0.10	43.6 ± 8.3	22.9 ± 2.6	5.4 ± 0.7	66.2 ± 10.2
LFA (left femoral artery)	3.68 ± 0.98	1.42 ± 0.33	153.1 ± 47.3	32.4 ± 8.6	9.9 ± 2.3	28.4 ± 5.7
HPV (hepatic portal vein)	10.9 ± 6.5	19.6 ± 20.4	369 ± 320	96.2 ± 56.8	136 ± 142	64.6 ± 7.6

[a] Only four animals were included in the calculation of the HPV group because GTN could not be detected in one of the animals.
[b] Results of multiple-comparison test: HPV > LFA = LJV = LFV.
[c] Results of multiple-comparison test: LJV = HPV = LFV > LFA.

our laboratory (22,23). The results suggest that multiple metabolic pathways for GTN metabolism may be present in the liver (22) and that different isozymes of glutathione *S*-transferase are capable of forming one GDN preferentially (23). Thus, the observed unique GDN formation ratio in rat livers may be due to a different distribution of various enzymes, or isozymes, when compared to other species.

In this study, the leg of the rat also demonstrates significant GTN extraction and metabolism ($F = 0.375$). Cossum *et al.* (8) also reported significant uptake of GTN in the legs of sheep, with the availability ranging from 0.12 to 0.29. When LFA infusions were administered, the GDN ratio obtained was significantly lower than the ratio obtained after LFV infusions. This suggests that GTN metabolism in the leg yields a GDN ratio less than 4:1 (the resultant GDN ratio from LFV infusions). Moreover, the LFA infusion studies yielded the lowest percentage of GTN clearance that could be accounted for by the formation of GDNs. These results could be due to high sequential metabolic activity, most probably to the mononitrate metabolites, for the GDNs in the leg.

Fung *et al.* (5) reported significant uptake of GTN by various blood vessels after bolus injections into the vasculature. Furthermore, the vascular GTN uptake is more extensive in venous tissues than in arterial tissues. Cossum *et al.* (8) reported that the sheep aorta has a very low GTN extraction ratio of 1 to 2%. However, no estimate of the venous sheep uptake was determined, although it is generally believed that GTN exerts its action predominantly on the venous tissues (24). Here, the venous GTN uptake was examined by comparing the LFV and LJV infusions. The segment of venous tree between these two infusion sites is represented mainly by the inferior vena cava. The extraction of GTN by the venous bed is approximately 20%, a higher value than that reported for the sheep aorta. Previously, Bennett *et al.* (25) reported a very specific 1,2-GDN formation from GTN by hemoglobin and myoglobin, with ratios of 10:1 and 4:1, respectively. Therefore, the high specificity for 1,2-GDN formation from the intravenous infusions in this study may result from blood metabolism. Hence, the role of blood metabolism may be more important than what was reported earlier, where metabolism in blood was estimated as only 1% of the total-body clearance of GTN (6). On the other hand, Slack *et al.* (26) recently reported a very specific 1,2-GDN formation (8:1) in rabbit aortic strips that were nontolerant to nitrates. In another rat study, homogenates of the arteries and veins generated GDN ratios of approximately 4:1 and 2:1, respectively (27). This indicates that blood vessels may also possess the capability to form 1,2-GDN preferentially. In addition, it has been reported that in tolerant tissues, the 1,2-GDN formation decreased more extensively than the 1,3-GDN formation (26–28). This leads to the suggestion that the 1,2-GDN formation pathway may in fact be the pharmacologically effective pathway. It is tempting to speculate that the organ or tissue which is responsible for the highly selective GDN formation in LFV and LJV infusions may also represent the pathway that is responsible for generating the effects of GTN on the venous tree.

Previously, Noonan and Benet (10) reported variable GDN ratios as a function of GTN dosing route in healthy human volunteers. Intravenous infusions resulted in the highest GDN ratio (about 8:1). The transdermal and sublingual preparations yielded intermediate ratios (about 4:1 and 5:1, respectively), while oral dosing resulted in the lowest GDN ratio (2:1). The results for LFV and HPV infusions in this study with rats are consistent with the trend observed in the previous human study—i.v. infusions yielding the highest GDN ratio and oral doses yielding the lowest ratio—although the values are different, probably due to interspecies differences. Therefore, this study supports the hypothesis that the differences in GDN ratios observed in the previous human study are a function of the route of administration.

It is important to mention that many of the pharmacokinetic calculations in this study assume linearity in GTN and GDNs disposition. We have performed GDN infusions in rats (12) to show linearity in the pharmacokinetics of both 1,2- and 1,3-GDN with increasing infusion rates at GDN concentrations similar to those observed here. Moreover, there was no noticeable interaction between the GDNs. Although 1,2-GDN seemed to possess a higher clearance value than 1,3-GDN, it also possesses a higher volume of distribution. As a result, the resultant half-lives and mean residence times appeared to be similar. Since the elimination half-lives of both GDNs are similar, it is possible to conclude that the observed GDN ratio is a reflection of the formation ratio, and not a function of differences in elimination kinetics for the GDNs.

In summary, intravascular infusion at various sites—though at the same rate—produce significantly different GTN and GDN concentrations. Moreover, the resulting ratios of GDN formation are also different. The liver, the leg, and the veins are all organs that can extract and metabolize GTN from the bloodstream, and they possess different patterns of dinitrate formation.

ACKNOWLEDGMENTS

This work was supported in part by NIH Grant HL32243. The authors thank Marion Laboratories, Kansas City, MO, for graciously supplying pure samples of the dinitrate metabolites and Ms. Lolin Ip and Ms. Elaine K. Chan for their excellent technical assistance.

REFERENCES

1. H.-L. Fung, H. Ogata, A. Kamiya, and G. A. Maier. Pharmacokinetics of nitroglycerin after parenteral and oral dosing in the rat. *J. Pharm. Sci.* 73:873–879 (1984).
2. E. F. McNiff, A. Yacobi, F. H. Young-Chang, L. H. Golden, A. Goldfarb, and H.-L. Fung. Nitroglycerin pharmacokinetics after intravenous infusion in normal subjects. *J. Pharm. Sci.* 70:1054–1058 (1981).
3. P. K. Noonan, R. L. Williams, and L. Z. Benet. Dose dependent pharmacokinetics of nitroglycerin after multiple intravenous infusions in healthy volunteers. *J. Pharmacokinet. Biopharm.* 13:143–157 (1985).
4. P. A. Cossum and M. S. Roberts. Metabolite inhibition of nitroglycerin metabolism in sheep tissue homogenates. *J. Pharm. Pharmacol.* 37:807–809 (1985).
5. H.-L. Fung, S. C. Sutton, and A. Kamiya. Blood vessel uptake and metabolism of organic nitrates in the rat. *J. Pharmacol. Exp. Ther.* 233:334–341 (1984).
6. P. K. Noonan and L. Z. Benet. Formation of mono- and dini-

trate metabolites of nitroglycerin following incubation with human blood. *Int. J. Pharm.* **12**:331–340 (1982).
7. P. A. Cossum and M. S. Roberts. Nitroglycerin disposition in human blood. *Eur. J. Clin. Pharmacol.* **29**:169–175 (1985).
8. P. A. Cossum, M. S. Roberts, A. J. Galbraith, and G. W. Boyd. Distribution and metabolism of nitroglycerin and its metabolites in vascular beds of sheep. *J. Pharmacol. Exp. Ther.* **237**:959–966 (1986).
9. L. J. Ignarro, H. Lippton, J. C. Edwards, W. H. Baricos, P. J. Kadowitz, and C. A. Gruetter. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside, and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* **218**:739–749 (1981).
10. P. K. Noonan and L. Z. Benet. Variable glyceryl dinitrate formation as a function of route of nitroglycerin administration. *Clin. Pharmacol. Ther.* **42**:273–277 (1987).
11. F. W. Lee, N. Watari, J. F. Rigod, and L. Z. Benet. Simultaneous determination of nitroglycerin and its dinitrate metabolites by capillary gas chromatography with electron capture detection. *J. Chromatogr.* **426**:259–266 (1988).
12. D. T.-W. Lau, M. Gumbleton, C. Labisch, and L. Z. Benet. Pharmacokinetic studies of 1,2- and 1,3-glyceryl dinitrates in the rat. *Biopharm. Drug Dispos.*, **12**:215–222 (1991).
13. J. H. Zar. *Biostatistical Analysis*, Prentice Hall, Englewood Cliffs, NJ, 1974.
14. U. Thadani and T. Whitsett. Relationship of pharmacokinetic and pharmacodynamic properties of the organic nitrates. *Clin. Pharmacokinet.* **15**:32–43 (1988).
15. J. F. Brien, B. E. McLaughlin, S. M. Kobus, J. H. Kawamoto, K. Nakatsu, and G. S. Marks. Mechanism of glyceryl trinitrate induced vasodilation. I. Relationship between drug biotransformation, tissue cyclic GMP elevation, and relaxation of rabbit aorta. *J. Pharmacol. Exp. Ther.* **244**:322–327 (1988).
16. G. S. Marks, B. E. McLaughlin, H. F. MacMillan, K. Nakatsu, and J. F. Brien. Differential biotransformation of glyceryl trinitrate by red blood cell-supernatant fraction and pulmonary vein homogenate. *Can. J. Physiol. Pharmacol.* **67**:417–422 (1989).
17. T. Winsor and H. J. Berger. Oral nitroglycerin as a prophylactic antianginal drug. Clinical, physiologic, and statistical evidence of efficacy based on a three-phase experimental design. *Am. Heart J.* **90**:611–626 (1975).
18. P. K. Noonan and L. Z. Benet. The bioavailability of oral nitroglycerin. *J. Pharm. Sci.* **75**:241–243 (1986).
19. D. K. Yu, R. L. Williams, L. Z. Benet, E. T. Lin, and D. H. Giesing. Pharmacokinetics of nitroglycerin and metabolites in humans following oral dosing. *Biopharm. Drug Dispos.* **9**:557–565 (1988).
20. E. Nakashima, J. F. Rigod, E. T. Lin, and L. Z. Benet. Pharmacokinetics of nitroglycerin and its dinitrate metabolites over a thirtyfold range of oral doses. *Clin. Pharmacol. Ther.* **47**:592–598 (1990).
21. N. H. Lee: The metabolism of glyceryl trinitrate by liver and blood from different species. *Biochem. Pharmacol.* **22**:3122–3124 (1973).
22. D. T.-W. Lau and L. Z. Benet. Differential formation of dinitrate metabolites from glyceryl trinitrate in subcellular fractions of rabbit liver. *Biochem. Pharmacol.* **38**:543–546 (1989).
23. D. T.-W. Lau and L. Z. Benet. Nitroglycerin metabolism in subcellular fractions of rabbit liver: Dose dependency of glyceryl dinitrate formation and possible involvement of multiple isozymes of glutathione S-transferases. *Drug Metab. Dispos.* **18**:292–297 (1990).
24. H. Kinadeter, J. Holtz, A. Kolin, and E. Bassenge. Different threshold dosages of nitroglycerin are required to induce venous pooling, afterload reduction or coronary dilation in conscious dogs. *Eur. J. Physiol.* **379** (Suppl.):R11 (1979).
25. B. M. Bennett, S. M. Kobus, J. F. Brien, K. Nakatsu, and G. S. Marks. Requirement of reduced, unliganded hemoglobin and myoglobin-mediated biotransformation of glyceryl trinitrate. *J. Pharmacol. Exp. Ther.* **237**:629–635 (1986).
26. C. J. Slack, B. E. McLaughlin, J. F. Brien, G. S. Marks, and K. Nakatsu. Biotransformation of glyceryl trinitrate and isosorbide dinitrate in vascular smooth muscle made tolerant to organic nitrates. *Can. J. Physiol. Pharmacol.* **67**:1381–1385 (1989).
27. H.-L. Fung and R. Poliszczuk. Nitrosothiol and nitrate tolerance. *Z. Kardiol.* **75** (Suppl. 3):25–27 (1986).
28. B. M. Bennett, D. C. Leitman, H. Schroder, J. H. Kawamoto, K. Nakatsu, and F. Murad. Relationship between biotransformation and cyclic GMP accumulation in various cultured cell lines. *J. Pharmacol. Exp. Ther.* **250**:316–323 (1989).